Changes in song repertoire and song rate of the pied flycatcher (*Ficedula hypoleuca*) by food manipulation – a field experiment

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Acknowledgments

Philosophically, I have come to think about the last half decade of my life as a journey. At first, it was a true leap of faith: I didn't know anyone, and I didn't know where the hell I was going. But little by little, I found my feet and I found my pace. And now, I couldn't be happier with the path that I have chosen! One journey has come to the end, but I am at the beginning of everything else.

First, I wish to express a warm thanks to my supervisor Helene Marie Lampe for offering me this thesis and helping me get through it. Thank you for giving heed to my pleasures and displeasures alike! Your patience and guidance made all the difference.

Sincere thanks to Ane Eriksen for all your assistance both in and out of the woods – you're a gem and a joy to work with!

Special thanks to

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- ✓ All the friends that I have made during my time at the Institute of Biology. I owe you for catchy phrases such as "Just keep swimming, just keep swimming, swimming, swimming…what do we do? We swim!!!" and a warm-hearted presence that has made tough times endurable and high times even more memorable.

Thank you, Dad, for visiting me in the field and for all those 1.000.000 phone calls coming from the long and winding road, letting me know that you were thinking of me!

Thank you, Mom, for housing and feeding me and helping with everything these last 6 years. I am forever grateful for all that you have done for me and hope to keep making you proud.

And thank you, Linda, for believing in me and walking the path alongside me. Your love and affection is a true blessing in my life.

Abstract

The nutritional stress hypothesis formulated by Nowicki *et al.* (1998) is based on the findings that brain structures underlying song learning in songbirds largely develop during the first few months post-hatching; a period when nestlings are most likely to suffer nutritional and other stresses that can have detrimental and lasting effects on the expression of song behaviour in close-ended learners. However, some bird species are considered to be open-ended learners, meaning that they have the ability to acquire and develop new songs every spring. Song acquisition throughout adulthood could compensate for any deficits due to stress during early development. I therefore tested the nutritional stress hypothesis by supplying mealworms to a group comprised of yearling and adult male pied flycatchers (*Ficedula hypoleuca*) during the breeding season of 2007. Song repertoires and song rates were of equal size in experimental males and controls prior to experimentation. Contrary to expectations, I observed a non-significant decrease in song repertoires for both groups and an increase in song rates for both groups, but significantly only for controls. Thus, treatment had no effect on song figure acquisition. However, I found that experience had a significant effect. Interestingly, experienced males who attained a mate were among those few individuals whose song repertoires increased. Maybe the bad weather conditions in 2007 gave experienced, high quality males that already knew the area an extra advantage when experimentally fed which the poorer quality, inexperienced males could not take advantage of.

Contents

Introduction

For several decades, bird song has been the focal point of extensive behavioural studies (Catchpole & Slater 1995). Many of these studies have taken an ultimate perspective, in the sense of asking *why* birds sing the way they do in terms of selective forces that have shaped the expression of this behaviour (reviewed in Searcy & Yasukawa 1996). The two main functions of birdsong are male-male competition and female choice (Catchpole & Slater 1995). In the context of mate choice, female birds very commonly exert a preference for complex song repertoires and elevated song rates (Searcy & Yasukawa 1996). Theoretical models point out that such female preferences could evolve if the females, by mating with males that have preferred song characteristics, obtain either direct benefits, such as better parental care or access to a better territory, or indirect benefits in the form of "good genes" for her offspring (Kirkpatrick & Ryan 1991).

Experimental manipulations of male singing behaviour with food have shown that males singing at a high rate were more attractive to females than males singing at low rates. Hence, males singing at high rates had a better mating success than control males (Gottlander 1987; Alatalo *et al.*1990). Song output is thought to be costly for the bird in terms of maintaining a balanced energy budget; time spent singing cannot be spent foraging and vice versa, hence the bird has to divide its time into either activity. But unlike song output, which may or may not be costly for the bird (Ward *et al.* 2004), song complexity is hard to explain as a reliable indicator of male quality because it is not apparent why complex songs would be more costly to produce than simpler songs (Nowicki & Searcy 2004).

Song complexity may reflect the quantity of learning in males and is measured either as the number of song types or syllable types that the male is able to sing (Nowicki *et al.* 2002). Hasselquist *et al.* (1996, but see also Hasselquist (1998)) found that extra-pair copulations (EPCs) in socially polygynous great reed warblers (*Acrocephalus arundinaceus*) are more commonly achieved for older, more experienced males who have larger syllable repertoires and also claim the best territories. Male common starlings (*Sturnus vulgaris*) with long song bouts and large song repertoires are paired earlier than males with small repertoires; this has been seen both in the field, where male arrival date was a confounding factor, and in the laboratory, where male arrival date was under the researchers' control (Eens *et al.* 1991).

Correlations between male song quality and parental abilities have been found in the sedge warbler (*Acrocephalus schoenobanus*) (Buchanan & Catchpole 2000), but such correlations are otherwise rare (Mounjoy & Lemon 1997; Nowicki *et al.* 2000; Rinden *et al.* 2000).

According to Nowicki *et al.* (1998), how well a male songbird sings – reflected in repertoire size or in other learned features of the male's singing behaviour, such as fidelity to a local song structure or "dialect" (Nowicki *et al.* 2002) – provides an honest indicator of quality because the timing of the development of brain structures mediating learning corresponds to a period in development during which young songbirds are most likely to undergo nutritional and other types of stress. This nutritional stress hypothesis, also known as the developmental stress hypothesis, predicts that stress experienced during 'sensitive stages' will impair brain development and the subsequent song learning and expression of song. Having "good genes" may act as a buffer to a certain extent, and reduce the impact of developmental stress. The hypothesis's key prediction is that the size of an individual's song repertoire is being determined at the nestling stage. Depending on how well or how poorly fed chicks are in a period critical for brain development, the song-control centres that influence adult song will develop differentially. As such, individual song repertoire sizes will depend on rearing conditions and the ability to cope with stressors early in life.

Some species of songbird, such as the common starling (Eens *et al.* 1991) and the pied flycatcher (*Ficedula hypoleuca*) (Espmark & Lampe 1993), are considered to be open-ended learners, meaning that they have the ability to acquire and develop new songs each spring (Nowicki *et al.* 1998). Repertoire sizes have been shown to grow with advancing age (Espmark & Lampe 1993; Lampe & Espmark 1994). Nowicki *et al.* (1998) state that if a species is capable of open-ended learning, then its song learning abilities may be contingent not only on the nutritional state of the young bird in its first year of life, but also on nutrition or other factors that exert an influence across the lifetime of the individual.

Based on an earlier assumption that song repertoire did not influence female mate choice in pied flycatchers (Alatalo *et al.*1986), Gottlander (1987) performed a field experiment where a group of pied flycatchers were fed mealworms in order to determine whether their song rate changed. If a high song rate by any likelihood could compensate for a poor song repertoire (Catchpole *et al.* 1984), then the males' song rate could be used as a cue by the females in

mate choice. Pied flycatchers sing at a lower rate in cold weather, but artificial feeding increased the rate to a level normal for good weather (Gottlander 1987). Song rate may be correlated with food availability within any given territory because song rate increased with higher temperature and thereby increased insect motility (Gottlander 1987). A study resembling that of Gottlander (1987) was conducted by Alatalo *et al.* (1990), who manipulated male pied flycatchers´ singing rates in the wild by providing mealworms in periods of cold weather and checking the males' mating order. This study indicated that experimental males were more successful in attracting females due to their increased singing rate (Alatalo *et al.* 1990).

Lampe & Sætre (1995) experimentally demonstrated that, when given the choice between a male with a small repertoire and less versatile song and another male with a large repertoire and more versatile song, female pied flycatchers prefer males having larger song figure repertoires and more versatile song. Other male characteristics that could affect the experiment, such as plumage colour, were similar in the two males (Lampe & Sætre 1995). The benefits of this apparent female preference for male song complexity are not known (Rinden *et al.* 2000). One potential benefit could be that males singing complex songs are of higher quality, endowed with more experience, brighter plumage, high body weight and better condition (Lampe & Espmark 1994). Males in better condition can afford to spend more time and energy on singing than males in poorer condition (Nowicki *et al.* 2002).

The aim of this study is to test the effect of supplemental food on song repertoire sizes of pied flycatchers; a species considered to be an open-ended learner (Eens *et al.* 1991; Espmark & Lampe 1993; Nowicki *et al.* 1998). By supplying mealworms to one group of males, and keeping another group of males as controls, I want to examine to which extent feeding conditions early in the breeding season can have an effect on male singing behaviour. I predict that good feeding conditions will result in larger song repertoires and higher song rates, and that these results may be obtainable within one breeding season.

Material and methods

Study species

The pied flycatcher is a small passerine bird averaging 12-13 g in body weight and 12-13.5 cm in length (Lifjeld 1994; Lundberg & Alatalo 1992). It only stays in the north for the spring and the summer (Lundberg & Alatalo 1992), and otherwise spends its time migrating to and fro the wintering grounds in West Africa, where it also spends a considerable time out of the year. Males frequently return to the same nesting grounds they used a year ago, whilst females are not so true to old nesting areas (Lampe, pers. com.). Female and male plumage is sexually dichromatic. Pied flycatchers depend on natural or man-made nest cavities, and are therefore found breeding throughout Norway apart from the most exposed coastal parts and in high altitudes (Lifjeld 1994), where nesting trees and key prey such as caterpillars for the nestlings is in poor supply. Flycatchers are – as their name proclaims – aerial insectivores able to catch insects while on the wing. They show a preference of deciduous forest over coniferous forest (Lifjeld 1994), but will breed at any place where natural nest sites or good quality nest boxes are available. Male pied flycatchers actively use song in mate attraction and acquisition (Eriksson & Wallin 1986), after which singing activity usually decreases significantly or subsists entirely (Gottlander 1987). However, some males exhibit polyterritorial behaviour (10-20% in the Oslo area (Lifjeld 1994)) by claiming a second nest box and sing in order to attract a secondary female. Polygynous males usually only assist in feeding the primary brood (Lifjeld & Slagsvold 1989).

Field site

The field site was a typical habitat for pied flycatchers situated at Sinober (59°59'N, 10°38'E) in Sørkedalen, near Oslo. The site is characterized as a mixed coniferous forest (Lampe *et al.* 2007). Nest boxes have been erected on tree trunks and hang approximately 50 m apart. Males breeding at Sinober are thus prospective to get neighbours on all sides (Rinden *et al.* 2000). During the breeding season (end of April-middle of July), males arrive approximately a week earlier than the females from the wintering grounds in Africa. Upon arrival, the males claim a nesting site and start to sing vigorously to attract a mate. All nest boxes within the field site were checked daily to monitor arrivals and the onset of nest building. Females immediately start nest building, which indicates when the males acquired a mate.

Study subjects

For my study, we recorded a total of 20 male pied flycatchers during the period from 28th of April 2007 to 8th of June 2007, but we did not record song during heavy rainfall. Monthly precipitation in May 2007 was 164% of normal value, and 205% of normal value in June 2007 (The Norwegian Institute of Meteorology, www.met.no). Most female pied flycatchers arrive at night (Gottlander 1987), so recordings were made in the period in which males are singing actively, approximately between 6:30 a.m. and 1:00 p.m. We used a combination of Sony cassette recorders (TC-D5M and TC-D5 PRO) and a Sound Devices 702 recorder equipped with headphones and Telinga parabolic microphones. Every male was subjected to two recordings, the first on arrival and the second approximately a week later. The time and location for every recording was logged, whereupon we made efforts to record at least 25 consecutive strophes for song analysis. When the first recording was concluded, the males were trapped into the nest boxes by means of a trap door and often using playback of male pied flycatchers as a lure. The male birds were age-assessed as being juveniles (one year old) or adults (two years old or more) on the basis of the amount of white and wear on the tip of the outermost greater wing covers (Svensson 1992). Adults can also be identified by the colour of their upper mandible, which is black or almost black (Svensson 1992). Age assessment was uncertain for two males, and these individuals were therefore not classified conclusively as either adult or juvenile. Plumage colour was determined by using the colour scale set by Drost (1936); males in group 1 being bright black and white and males in group 7 being female-like brown. Half- and quarter-scores were used for intermediate types (Sætre *et al.* 1994; Dale *et al.* 2002).

Upon capture, morphological data was gathered from every male: the length of the wing, once flattened and straightened, was measured to the nearest 0.5 mm by aid of a ruler. Tarsus length (with toes bent measured from the extreme bending point at the inter-tarsal joint) and forehead patch height from bill was measured to the nearest 0.1 using a sliding caliper. Body weight was measured to the nearest 0.1 g with a Pesola spring balance. Every male was given individual identification codes with one serial coded aluminium ring, two coloured plastic rings, and plumage markings with a red marker. I divided body weight on tarsus length as a measure of body condition because, unlike wing length, tarsus length is more independent of age (Dale *et al.* 2002). In addition, tarsus length is a trait known to be strongly affected by developmental conditions (Doutrelant *et al.* 2000).

The birds were divided into two different groups; half of the individuals were fed mealworms (*Tenebrio molitor*) that we supplied in the immediate vicinity of the nest boxes over the course of a week, and the other half of the individuals were not fed and thus assigned as controls. Male pied flycatchers defend a rather small territory, and most territorial fights have therefore been shown to take place near the defended nest site (median 10 m, von Haartman 1956a), while foraging areas can be at some distance from the nest and are often overlapping with other individuals (von Haartman 1956a).

By the time the males were to be re-recorded, some had become paired. Paired males who had ceased to sing, had to be induced into doing so by mate removal, unless they were polyterritorial. The females can be captured inside the nest boxes because female pied flycatchers build the nest single-handedly, and make recurrent rounds back to her male's nest box in the process. Female birds were weighed and given individual identification codes with one serial coded aluminium ring and one coloured plastic ring. The females were kept in cages loosely covered by cloth to reduce visual input and stress, and given mealworms to eat *ad libitum*. With the female out of sight, the male pied flycatcher starts to sing again. At the end of the second recording, the females were freed to rejoin their mate and resume nest building.

Statistics

Song analysis was based on spectrograms created in Raven (Cornell Laboratory of Bioacoustics, version 1.2.2 for Mac OS X) by extracting 25 consecutive strophes from each of the two recordings and identifying the different song figures used. The following variables (as defined by Espmark & Lampe (1993)) were used in the song analyses:

a. Repertoire size, defined as the number of different figure types sung in each recording, early and late in the season, respectively. Total repertoire size is consequently defined as all song figure types contained in the sample of 2x25 strophes from every male.

b. Duration of the song strophe was measured on the spectrograms to the nearest 0.01 second.

c. Strophe diversity, which was calculated by dividing the number of different figure types in a strophe by the total number of figures in the strophe.

d. Song diversity, defined as the sum of figure types in a male's sample of strophes (i.e. song figure repertoire) divided by the total number of figures in the sample.

e. Song rate, defined as the number of songs sung per minute. Song rate was calculated by dividing 25 by the total time needed for each recording.

In order to analyse to what extent song figure types in a male's repertoire were the same before and after the experiment, I calculated an overlap index (OI) using a formula originally given by Schoener (1968)

$$1-\tfrac{1}{2}\,\Sigma\,|Pa_i - Pb_i|$$

Schoener devised the formula while investigating resource partitioning in *Anolis* lizards, but the formula is applicable for song analyses; Pa_i and Pb_i translates to the relative proportion of song figure type "i" in experimental stages a and b (Espmark & Lampe 1993).With increasing similarity between song prior to and after the experiment with regard to figure types, the index approaches the value 1, whereas increasing dissimilarity makes the index approach zero (Espmark & Lampe 1993).

Statistics were generated in R (version 2.6.2) and Statview. The song data was not normally distributed, so non-parametric methods were applied: The Spearman rank test was used to test for positive correlations between variables, without making any assumptions about the frequency distribution of the variables. The Wilcoxon paired rank test was applied as a non-parametric alternative to the paired Student's t-test. To test for differences between groups, such as age, treatment and experience, the Mann Whitney U-test was applied. Mating status as a grouping variable exceeded the criteria for testing with Mann Whitney U, so I used the Kruskal-Wallis one-way analysis of variance, which is an extension of the Mann-Whitney U test to 3 or more groups.

Results

Song variables

Prior to my experiment, song repertoires in both groups were similar (Fig. 1, Mann Whitney U-test; n = 20, U = 43.5, p = 0.62) and song rates were also similar in the two groups (n = 20, U = 43.0, p = 0.60). At the end of the experiment, song repertoires did not increase, if anything they decreased both in experimental males and in controls (Fig. 1, Wilcoxon paired rank test; n = 10, z = -1.58, p = 0.11 and n = 10, z = -0.63, p = 0.53, respectively). Song rates of experimental males did increase, but not significantly (z = -1.78, p = 0.074), while song rates of controls increased significantly (z = -1.99, p = 0.047) (fig. 1).

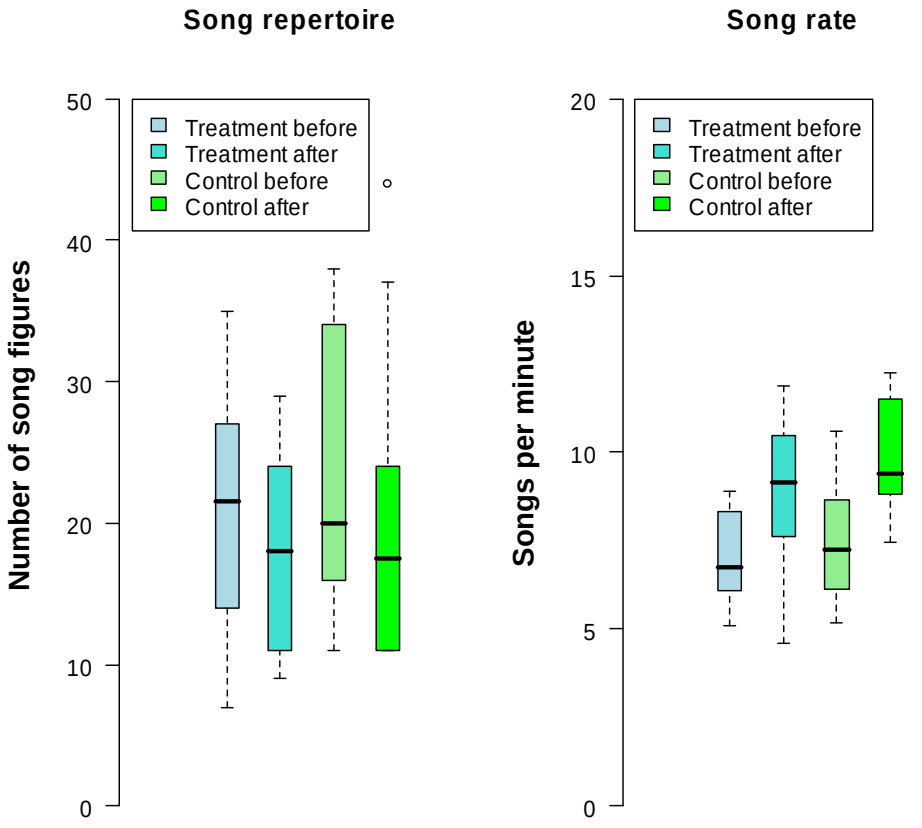

Figure 1
Plots showing the differences in song repertoires and song rates for experimental males (n=10) and controls (n=10) prior to and after the feeding experiment.

Strophe length and repertoire size was closely inter-correlated at the onset of the experiment (fig. 2a, Spearman rank test: $r_s = 0.66$, $p = 0.004$), but not after the experiment (fig. 2b, $r_s = 0.40$, $p = 0.08$).

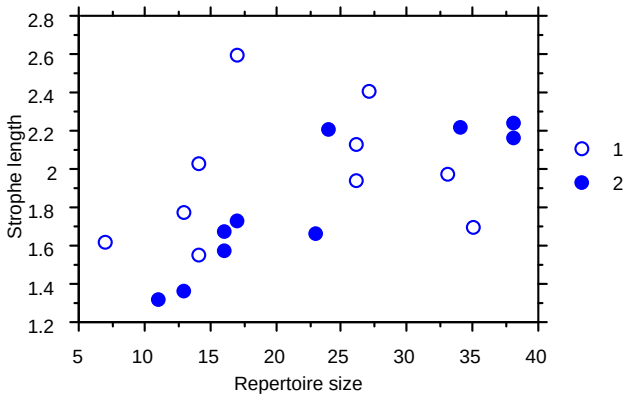

Figure 2a
Strophe length and repertoire size is inter-correlated prior to the experiment. Legend shows experimental males (1) and controls (2).

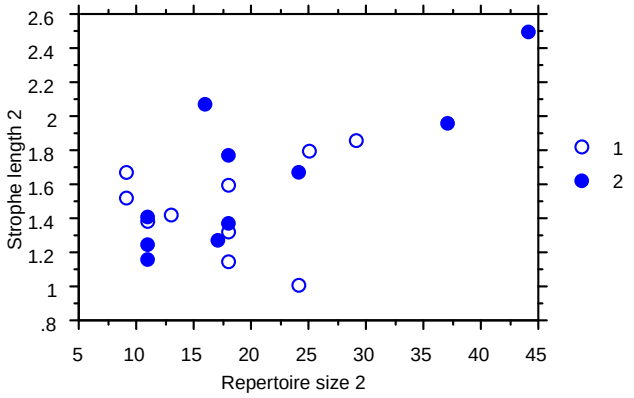

Figure 2b
Strophe length and repertoire size is not inter-correlated after the experiment. Legend shows experimental males (1) and controls (2).

When splitting males into groups based on treatment and mating status, the change in repertoire size and strophe length before and after the experiment was also significantly correlated (fig. 3, $r_s = 0.77$, $p = 0.0008$). The change in repertoire size and mean strophe length decreased in all birds save four: a paired control male, a paired control male who had to be induced to sing by mate removal, a polyterritorial control male and an unpaired experimental male.

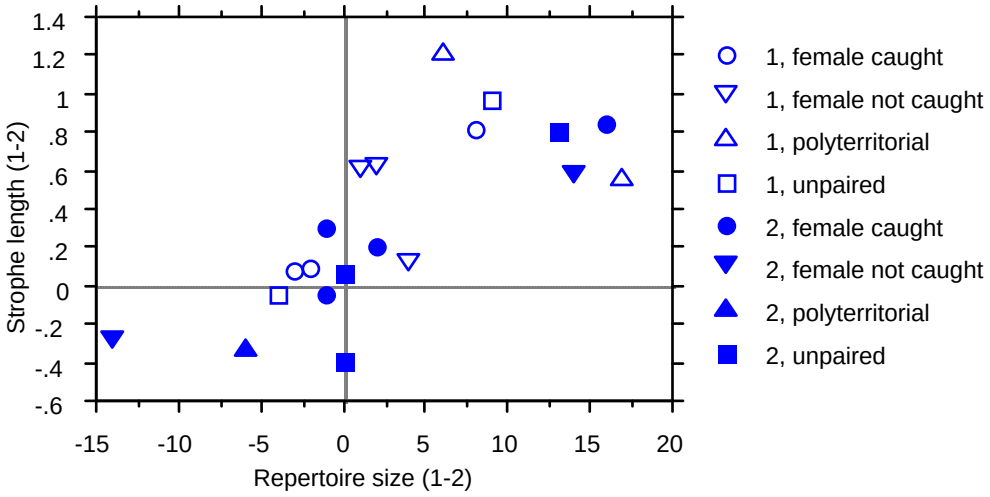

Figure 3
Points show changes in mean strophe length and song repertoire for experimental males (1) and controls (2), with emphasis on individual mating status. Points lying on the negative side of the x axis indicate higher values in the 2nd recording than in the 1st recording, in other words an increase in song repertoire and mean strophe length.

Song diversity and strophe diversity was marginally inter-correlated prior to the experiment (figure 4a, $r_s = 0.45$, $p = 0.051$) and significantly inter-correlated after the experiment (figure 4b, $r_s = 0.49$, $p = 0.032$).

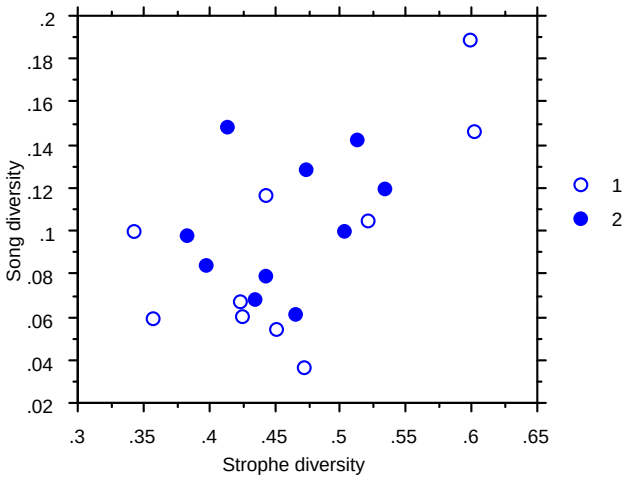

Figure 4a
Song diversity and strophe diversity prior to experiment is marginally inter-correlated. Legend shows experimental males (1) and controls (2).

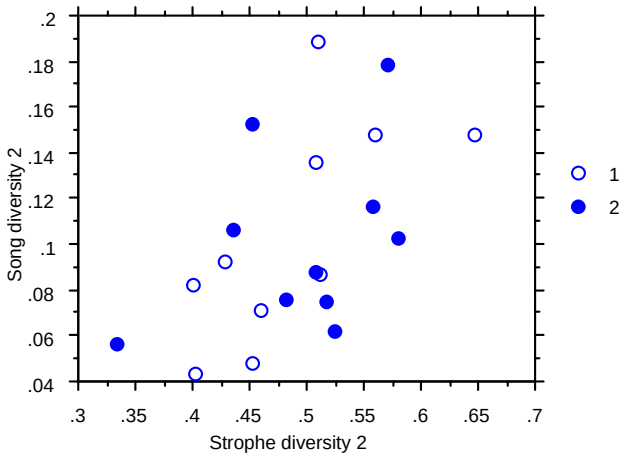

Figure 4b
Song diversity and strophe diversity is closely inter-correlated after the experiment. Legend shows experimental males (1) and controls (2).

Song diversity and repertoire size was closely inter-correlated both prior to (fig. 5a, $r_s = 0.86$, $p = 0.0002$) and after the experiment (fig. 5b, $r_s = 0.91$, $p < 0.0001$).

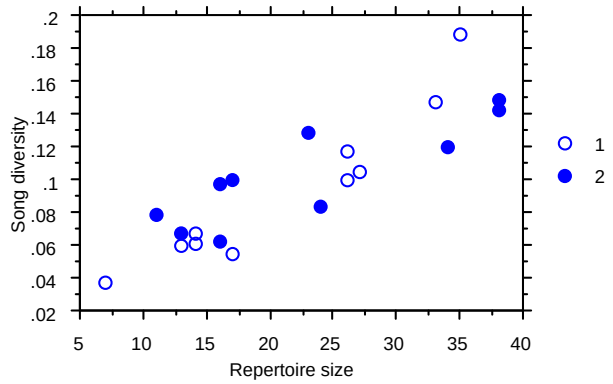

Figure 5a
Song diversity and repertoire size is closely inter-correlated prior to the experiment. Legend shows experimental males (1) and controls (2).

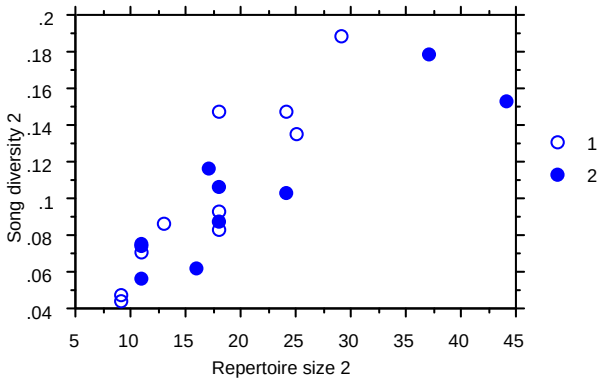

Figure 5b
Song diversity and repertoire size is closely inter-correlated after the experiment. Legend shows experimental males (1) and controls (2)

Song rate and song diversity was not significantly inter-correlated either before or after the experiment ($r_s = 0.22$, $p = 0.34$ and $r_s = -0.09$, $p = 0.68$, respectively). However, changes in song rate were significantly inter-correlated with changes in song diversity (fig. 6a, $r_s = 0.47$, $p = 0.039$) and marginally inter-correlated with changes in strophe diversity (fig. 6b, $r_s = 0.44$, $p = 0.054$).

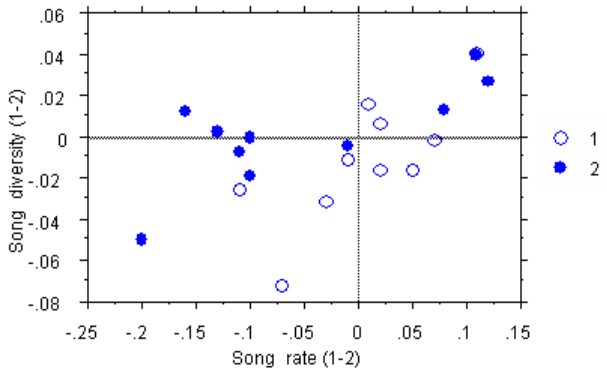

Figure 6a
Changes in song diversity and changes in song rate are correlated. Legend shows experimental males (1) and controls (2).

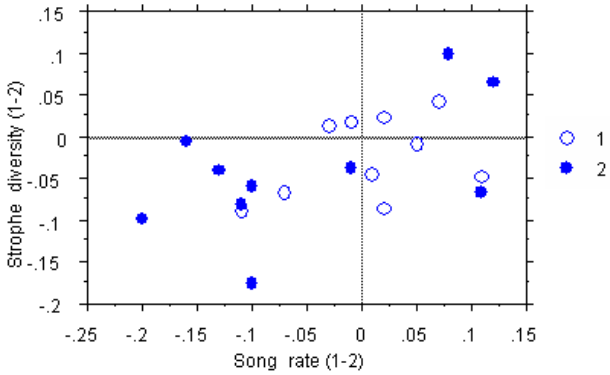

Figure 6b
Changes in strophe diversity and changes in song rate are marginally correlated. Legend shows experimental males (1) and controls (2).

Strophe diversity and strophe length was not significantly inter-correlated, neither prior to the experiment ($r_s = 0.17$, $p = 0.45$) nor after the experiment ($r_s = -0.09$, $p = 0.70$). Strophe diversity and repertoire size was not significantly inter-correlated before the experiment ($r_s = 0.41$, $p = 0.077$) and not after the experiment ($r_s = 0.35$, $p = 0.12$).

Males with uncertain age assessments were assigned to the age group they were most likely to be, and all song variables tested against age using Mann-Whitney U. Age was a significant variable on post-experimental song diversity ($U = 22.5$, $p = 0.04$), post-experimental repertoire size ($U = 22.0$, $p = 0.034$) and on changes in strophe diversity ($U = 23.0$, $p = 0.044$). Age was also a marginally significant variable on post-experimental strophe diversity ($U = 24.0$, $p = 0.052$), all other p-values > 0.21.

All song variables were Kruskal-Wallis-tested with mating status as a grouping variable, but mating status was non-significant (all p-values > 0.25). All song variables were then Mann Whitney U-tested with experience as a grouping variable. Experience is here defined as to whether males are returning to the same nesting grounds they used a year ago (n = 7), or if the study site is unfamiliar to them (n = 13).

Experience was marginally significant on pre-experimental song diversity ($U = 21.0$, $p = 0.052$) and significant on post-experimental song diversity ($U = 9.5$, $p = 0.0043$) and on the changes in song diversity ($U = 17.0$, $p = 0.024$). Experience was significant on post-experimental repertoire size ($U = 16.0$, $p = 0.02$) and marginally significant on the changes in repertoire size ($U = 21.5$, $p = 0.057$), all other p-values > 0.22.

Experience had a significant effect on changes in repertoire size (ANOVA, $F = 5.37$, $p = 0.039$, power = 0.56), whereas an interaction between experience and mating status ($F = 1.095$, $p = 0.38$, power = 0.22) or mating status alone ($F = 0.19$, $p = 0.89$, power = 0.077) had no significant effect on changes in repertoire size (fig. 7). However, all experienced males who attained a female (n = 5), increased in song repertoire size (fig. 7).

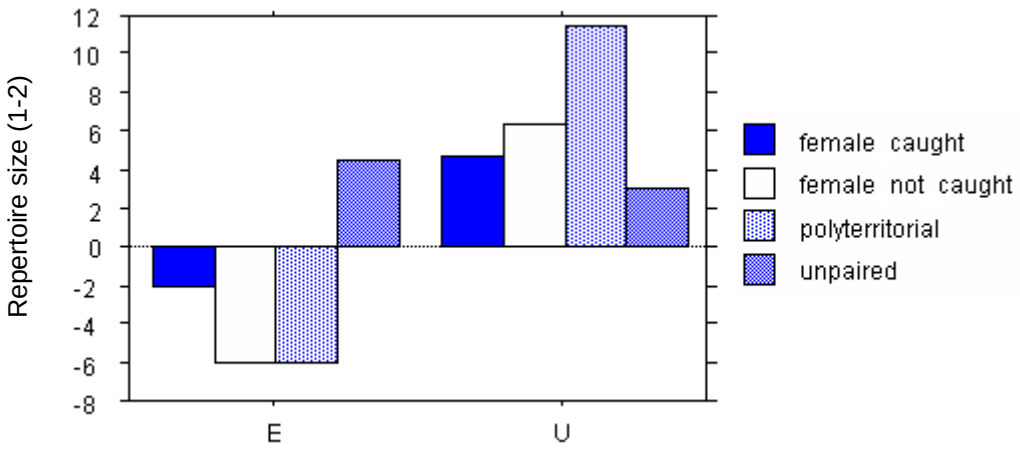

Figure 7
Bars show differences in repertoire sizes before and after the feeding experiment. Males are experienced (E; i.e. have bred at Sinober previously) or inexperienced (U; i.e. are first time breeders at Sinober).

Experience had a non-significant effect on changes in song rate (U = 36.0, p = 0.45). Neither experience (ANOVA, F = 0.415, p = 0.531, power = 0.090) nor mating status (F = 1.418, p = 0.285, power = 0.279) nor the interaction of experience and mating status (F = 0.681, p = 0.580, power = 0.151) had a significant effect on the changes in song rate (fig. 8). All males increased in song rate, save polyterritorial ones and inexperienced, unpaired males.

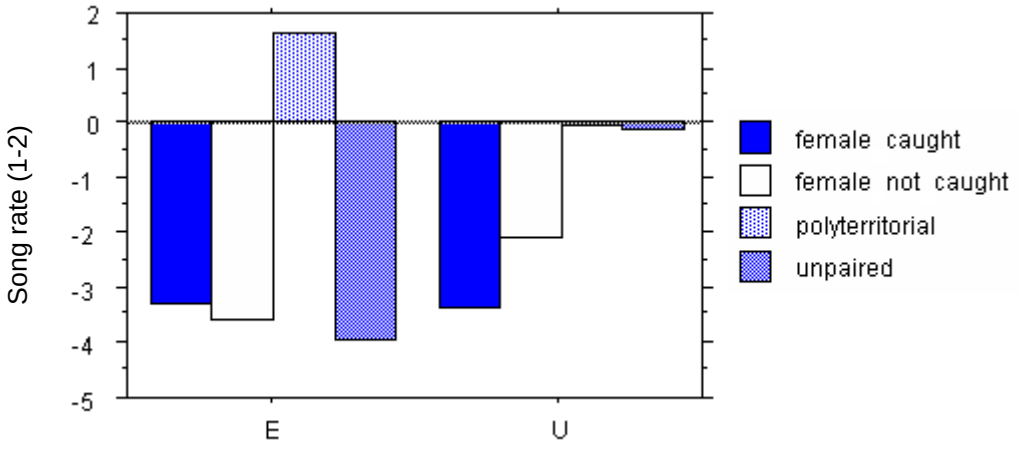

Figure 8
Differences in song rate before and after the feeding experiment for males with varying experience and mating statuses.

Experienced males increased their repertoire more than inexperienced, but not significantly (fig. 9, Mann Whitney U- test; n = 20, U = 21.5, p = 0.057).

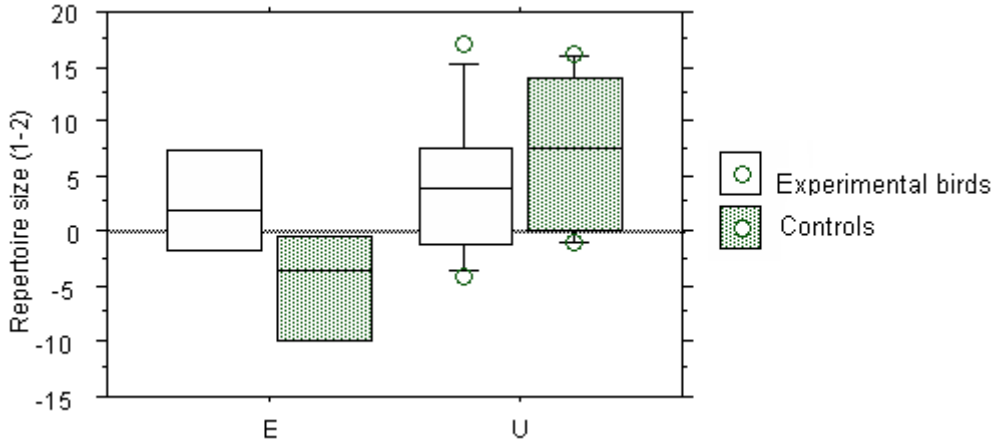

Figure 9
Changes in repertoire size in experienced vs. inexperienced birds, splitting males up by treatment

The overlap index (OI) for experimental males shows greater variance, but apart from this the groups did not differ in the way they added or removed song figures (fig. 10, Mann Whitney U-test: n = 20, U = 56.5, p = 0.59). The somewhat large OI of experimental males indicates relative stability in the use of song figure types, whereas the slightly smaller OI of controls indicates addition or removal of many song figure types. Controls possessed slightly larger repertoires in both recordings (29.4 song figure types) than experimental males (27.1 song figure types).

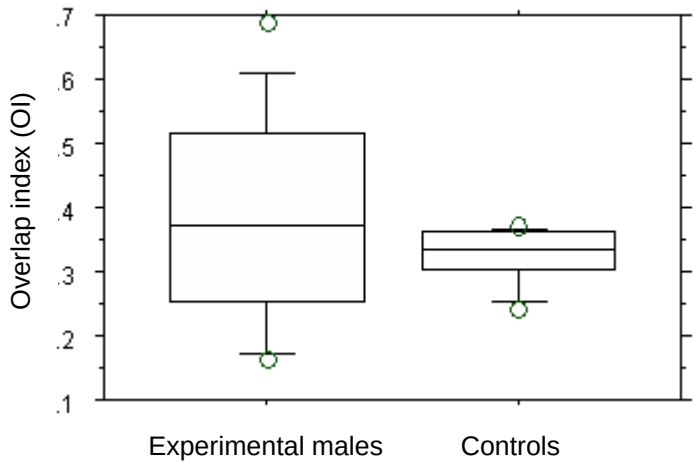

Figure 10
Overlap index (OI) for song figure types in experimental males and controls.

Experienced experimental males did not have significantly larger OI than experienced control males (fig.11), but only 7 individuals in this group is too few to get a meaningful statistical result.

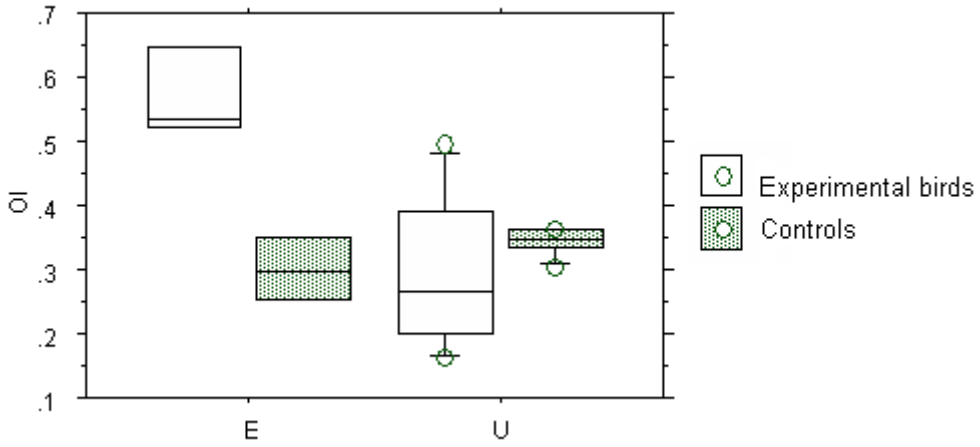

Figure 11
Overlap index (OI) for song figure types extracted from 2x25 song strophes in experimental males and controls, splitting males up by being experienced (n = 7) or inexperienced (n = 13).

There were no age differences within the experienced group of males that could explain the differences between experimental and control males (fig.12).

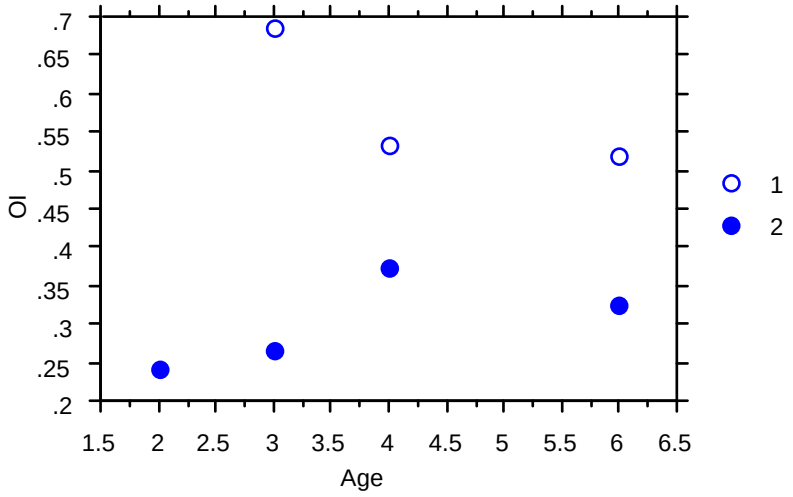

Figure 12
Overlap index (OI) for song figure types extracted from 2x25 song strophes in experimental males and controls, focusing solely on the individuals with previous experience (n=7).

Male characteristics

Experience was a significant variable on weight (U = 17.0, p = 0.024), wing length (U = 21.0, p = 0.048) and body condition (U = 14.5, p = 0.013). Age was a significant variable on experience (U = 2.0, p = 0.0003), body weight (U = 12.5, p = 0.005), wing length (U = 23.5, p = 0.045), body condition (U = 15.0, p = 0.008) and colour (U = 17.5, p = 0.014). Condition was a non-significant variable on treatment (U = 43.5, p = 0.62) and non-significantly inter-correlated with the changes in song rate (Spearman rank test, n = 20, r_s = -0.41, p = 0.07) and changes in strophe diversity (r_s = -0.43, p = 0.06). The relationship between several morphological variables was significant, such as for age and colour (r_s = -0.63, p = 0.0013), and colour and condition (r_s = -0.55, p = 0.017), or marginally significant, such as for colour and weight (r_s = -0.44, p = 0.053). Plumage colour was evenly spread in experimental males (3.95 ± 1.06) and controls (3.75 ± 1.52), but the variation was larger in the control group (fig. 13). Wing length was non-significantly larger in controls (80.4 ± 1.37 mm) than in experimental males (79.55 ± 1.90 mm) (fig.14, Mann Whitney U-test; n = 20, U = 29.5, p = 0.12).

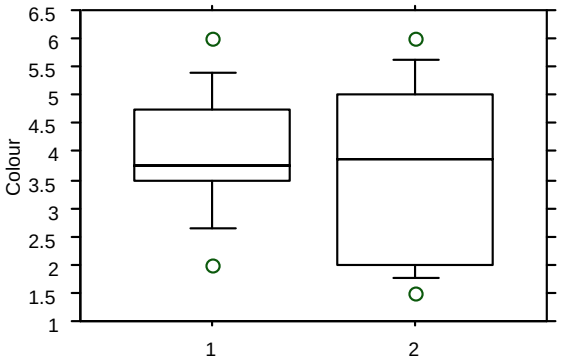

Figure 13
Plumage colour distribution in experimental males (1) and controls (2).

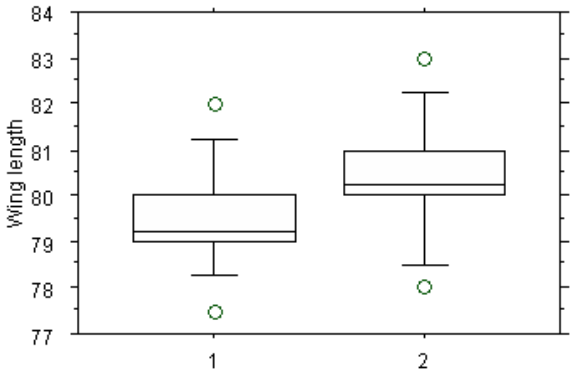

Figure 14
Distribution of wing length in experimental males (1) and controls.

A small fraction of the inexperienced males showed an increase in song repertoire, whereas the majority had smaller song repertoires and brownish plumage (fig. 15). Experienced males were darker in colour (Mann Whitney U-test, n = 20, U = 3.0, p = 0.0007), but colour and changes in repertoire sizes was not significantly inter-correlated (Spearman rank test; r_s = 0.39, p = 0.09).

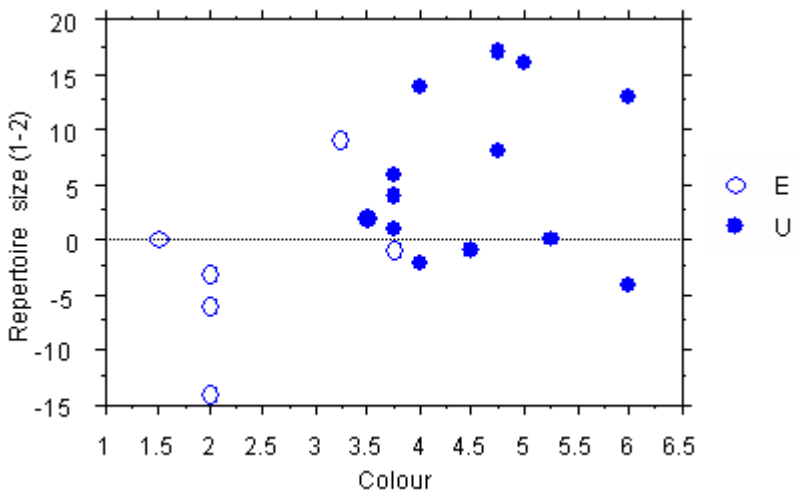

Figure 15
Points show differences in repertoire size before and after the feeding experiment, relative to the males' previous experience (E) or lack of experience (U) and individual plumage colour.

Song rate increased in all individuals save for one experienced and four inexperienced males (fig.16). Plumage colour and the changes in song rates was not significantly inter-correlated ($r_s = 0.41$, $p = 0.07$).

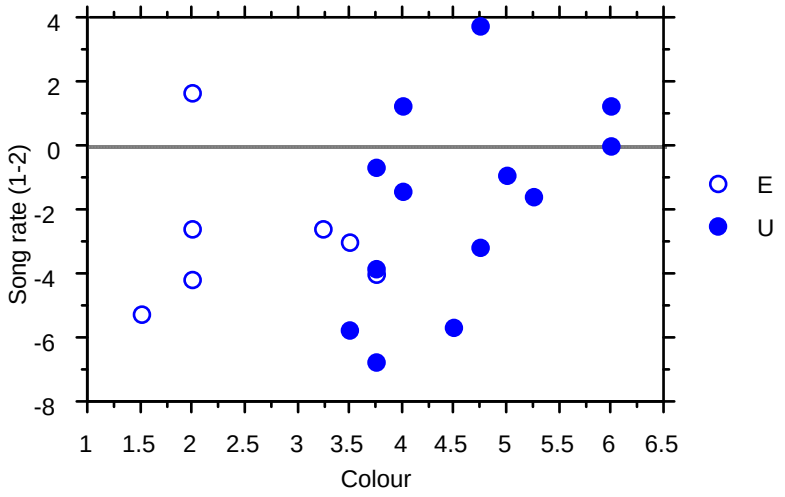

Figure 16
Points show differences in song rate before and after the feeding experiment, relative to the males' previous experience (E) or lack thereof (U) and individual plumage colour.

Discussion

Song repertoires in experimental and control males were of equal size prior to the feeding experiment, and the outcome was a non-significant decrease for both groups. Increase in mean strophe length and repertoire size was limited to four individuals, among these a single experimental, unpaired male. Thus, my first prediction stating that experimental males' song repertoire should increase as a result of the supplementary feeding is contradicted. The non-invasive nature of this experiment makes it difficult to know whether the food deployed was actually consumed by the experimental males or not. Following this argument, the males in my study may not have been sufficiently affected by treatment because of loss of the supplied food. The plastic lids protecting the food dishes sometimes gave way as a consequence of rain. In these cases the mealworms would drown in the dishes and swell out, plausibly either surpassing optimal prey size for the pied flycatchers or simply appear unappetizing for the birds. During downpours, food dishes risked getting knocked completely off the tree trunks, thereby loosing the food deployed that day. Great spotted woodpeckers (*Dendrocopus major*) were also periodically seen nearby the food dishes (pers. obs.), and so the degree of interspecific competition for food is a factor of unknown proportions. If competition with woodpeckers was so intense that it induced higher levels of stress in the birds, then this might have contributed to the decrease in song repertoires.

The males in my study may not have been sufficiently affected by treatment due to insufficient duration of experimentation. Food was supplied for a week and 2nd recordings carried out on the 7th day whenever possible, otherwise on any following day. It is conceivable that one week is too limited time for male pied flycathcers to acquire new song figure types. As Hultsch & and Todt (1998) point out, song acquisition depends on exposure variables: While the rate of song-type acquisition is only 30 % for songs experienced only 5 times, nightingales (*Luscinia megarhynchos*) imitate around 75 % of those song-types which they have heard 15 times (Hultsch & Todt 1998). In line with this, the males in my study should have been sufficiently exposed to neighbours' song over the course of a week, provided that neighbours were within hearing range. The limited increase of song repertoires might thus be due to the early pairing. Sinober is equipped with many nest-boxes, but males might per chance have been too far apart to hear neighbouring males from which they could have copied song elements. If males did not have time to sing for very long before they were

mated and consequently diminished in singing activity, then neighbour-copying would be minimal. Nevertheless, the increase in song repertoire size occurring in experienced males who had attained a female this season is an interesting finding.

Irrespective of manipulations of food availability, male pied flycatchers often show changes in their repertoire pre- and post-mating. After pairing, the males generally reduce their singing activity; strophes shorten and include fewer figures and/or figure types, song versatility increases but repertoire size decreases (Espmark & Lampe 1993). A previous study showed that some males retained most of their unmated repertoire when they were mated, whereas others changed to a completely new repertoire after they became paired (Espmark & Lampe 1993).

Lampe & Espmark (1994) concluded that older males in general have more complex songs than younger males. Repertoires of male great reed warblers also continue to change throughout adulthood, and generally increase with age (Hasselquist 1998). Other male characteristics, such as body size, plumage colour, breeding experience, singing behaviour and survival, have all been suggested to be good measures of male quality, and these characteristics also appear to be correlated with individual differences in the song pattern of pied flycatchers (Lampe & Espmark 1994). In my study, a significant correlation between repertoire size and colour was not observed, but having a bright plumage could possibly compensate somewhat for having a poorer song repertoire. When given a choice, females prefer males with brighter (black and white) plumage. This has been shown both in field and aviary experiments (Sætre *et al.*1994). Song figure repertoire, song versatility and male characters combined seemed to be the best measures of male quality in Lampe & Espmark's study area in 1994. At Sinober, there was a close inter-correlation between strophe length and repertoire size before the experiment begun, but this correlation had ended by the end the experiment. Individual male variation could have overruled the clear and segregated differences that we sought to obtain within the groups. If we assume that the generally larger repertoires of older and more experienced birds are a hallmark that these individuals are better endowed for acquiring new song types, experienced birds may have been under-represented in this study. In addition, it is difficult to sample total song figure repertoires because the males might possess many more song figures than what is actually being sung at any given moment. The actual sizes of the song repertoires can therefore have been under- or over-estimated.

However, this raises a new question: if having a large repertoire is attractive to females, why are males not using more of their repertoire at any given time?

Regarding overlap indexes, the two groups did not differ in the way that they added or removed song figures to their song repertoires. Compared to control males, experimental males had a large overlap index which implies that their repertoires remained fairly stable over time. Previous studies on song variation between breeding seasons found a high degree of stability in some individuals, whereas others were extremely innovative (Espmark & Lampe 1993). Perhaps change in song repertoire is not costly for the birds, but rather the maintenance of it? In the present study, some of the older males use their song repertoires in a very conservative manner and hence have a large OI. The underlying mechanism for stability in repertoires might be the supplementary food that these individuals received.

Song rates increased in both groups, but significantly only for controls. My second prediction, stating that song rates should increase in experimental males as a result of supplementary feeding, is therefore likewise contested. A comparison of methods with Gottlander (1987) and Alatalo *et al.* (1990) would be beneficial for my study, but duration of supplementary feeding is not specified in the aforementioned articles. Therefore, it is hard to tell how long supplementary feeding should have gone on for in order to observe the desired effect on experimental males. Independently of the extra food supplied, all males save polyterritorial ones increased in song rate. Lampe & Espmark (1994) observed that song rate was not correlated with male characters such as body size, plumage colour, breeding experience, singing behaviour and survival, but rather increased steadily during the breeding season; an increase that might be explained by food availability. Warmer weather is known to influence insect motility and, in turn, the birds' feeding and singing frequencies. This is reflected in several other studies (Gottlander 1987; Alatalo *et al.* 1990; Espmark & Lampe 1993). The apparent decrease in song rate for all polyterritorial males seems counter-intuitive and incompatible with the task of attracting a secondary female. Given the fact that Sinober is a relatively open forest, and that sound consequently travels farther than in a dense habitat (Lampe *et al.* 2007), males seeking secondary females may have to travel greater distances to keep out of earshot and subsequent harassment of his primary female (Slagsvold *et al.* 1992). Keeping a longer polyterritorial distance in unstable weather such as the one experienced in the springtime of 2007 could possibly be so energy-demanding that birds are left with little energy to sing. Another and more plausible explanation for low song rates in polyterritorial

males could be that the field site was devoid of available females late in the season, thereby causing a low motivation to sing.

Experience and age was a significant variable on weight, wing length and condition. In addition, age was a significant variable on plumage colour. Several morphological variables, such as colour, condition and weight were significant or nearly significantly inter-correlated. Wing length generally increases with age (Dale *et al.* 2002) and was non-significantly larger in controls than in experimental males, reflecting the finding that many individuals in the control group were older and more experienced birds. Lampe & Espmark (1994) have previously shown that repertoire size and song versatility correlates with male quality, measured as plumage colour, body condition and breeding experience. Plumage colour was not evenly spread in the two groups, as experimental males showed small variation and included more brown individuals than controls. In sum, the males participating in this feeding experiment followed general trends previously observed in other pied flycatcher populations.

Pied flycatchers hardly sing after pairing, but some males carried on singing after they were mated. Is it feasible that these males committed to a form of mate guarding? Forstmeier & Balsby (2002) studied dusky warblers (*Phylloscopus fuscatus*) because males of this species sing intensively after pairing and thus presented itself as a potential candidate for a so-called fertility announcement or male quality announcement hypothesized by Møller (1991). Total singing intensity in dusky warblers was observed to be clearly peaking during the egg-laying period, thus fitting expectations derived from the fertility announcement hypothesis, but mate guarding was hardly ever witnessed (Forstmeier & Balsby 2002). Mate guarding – in a strict sense – involves males actively following their mate, which has not been observed in pied flycatchers (Lampe, pers. com.), so this proposition probably serves as an *ad hoc* hypothesis at best. The bad weather may have forced females to forage further away from the nest box and to be absent for longer periods, thus inducing the males to sing. The effort taken in daily surveys may have rendered it possible to perform 2nd recordings at moments when paired males sang because their female was out of sight.

So how applicable is the nutritional/developmental stress hypothesis? Some bird species fit the criteria of the hypothesis well, given that song learning takes place in a limited phase of nestling development. In one experimental test of the developmental stress hypothesis, lab-reared swamp sparrows (*Melospiza georgiana*) were subjected to nutritional stress early in

their development, and this had a lasting effect on brain structure and copying accuracy of model songs compared with *ad libitum* controls (Nowicki *et al.* 2002). In addition, the volumes of two song-control regions in the brain, HVC (higher vocal centre) and RA (robust nucleus of the arcopallium), were significantly smaller in experimental males compared to controls (Nowicki *et al.* 2002). A more recent experimental test sought to test a second prediction, namely that song sparrows (*Melospiza melodica*) that had suffered nutritional stress early in their development could be impaired not only in song production but also in song perception later in life (MacDonald *et al.* 2006). Development of the song-control system could be impaired prior to the onset of song learning, and this could possibly affect both males and females (MacDonald *et al.* 2006). If overall reduction in brain size accounted for reduction in the size of the song-control system, effects of food restriction would be detectable throughout the song-control region. However, results showed that food restriction caused a specific decrease only in the volume of HVC for both males and females and moreover at an extremely young age, coinciding with the time when song sparrows begin to acquire song (MacDonald *et al.* 2006).

Spencer *et al.* (2003) found that nutritional stress suffered for 25 days significantly affected nestling growth and basal plasma corticosterone levels in zebra finch nestlings. Effects of nutritional stress also manifested themselves later in life on reduced strophe length, song complexity and peak frequency (kHz) reached in a song motif. Spencer *et al.* (2003) claimed this "the first empirical evidence to support the hypothesis that song complexity is a reliable indicator of early developmental stress, which can provide a basis for the evolution of female choice, based on this trait". Naguib *et al.* (2008) exposed non-domesticated female zebra finches to pairs of males that differed in the experimental brood size in which they had been raised. Under the assumption that experimental brood size would affect developmental conditions, Naguib *et al.* (2008) predicted that females should associate more with males that had experienced better developmental conditions and hence would be more sexually attractive. However, females did not spend significantly more time with males that came from relatively smaller brood sizes, and had elevated song rates and/or larger repertoires. One explanation could be that traits determining sexual attractiveness were either not sufficiently affected, or that the birds had compensated for deficits experienced during early development (Naguib *et al.* 2008). This contrasts with previous studies on developmental stress (Nowicki *et al.* 2000; Nowicki *et al.* 2002; Buchanan *et al.* 2003; Spencer *et al.* 2003; Zann & Cash

2007) but Naguib *et al.* (2008) argue that brood size manipulation is a comparatively weaker stressor than a directly imposed nutritional deficit.

In view of this, pied flycatchers might not be an ideal model for testing the developmental stress hypothesis in a strict sense, since this species can acquire song elements throughout adulthood. Any effects on a male's song characters due to nutritional stress during early development – which we did not impose – could be overcome by compensation later in life, which I tried to test. However, compensation as in my feeding trial did not seem to be enough. In the study of Espmark & Lampe (1993), acquisition of new song types was more pronounced from one year to another, whereby male pied flycatchers could change their song structure and pattern substantially between breeding seasons. This has also been observed in starlings (Eens *et al.* 1991), and individual differences in the song pattern of both species seem to be correlated with male age (Espmark & Lampe 1993). In other words, we know that pied flycatchers have the ability to learn new songs, but we do not know yet how "open-ended" these supposedly open-ended learners are. Refining the experimental design may be needed to find an interval of supplementary feeding that is optimal for my purposes. Also, we could hope for a larger sample size than 20 individuals. Studies such as the ones of Gottlander (1987) and Alatalo *et al.* (1990) showed that supplementary feeding influenced song rates of pied flycatchers in colder temperatures. If all my experimental males were in superior condition, this could explain why they showed so little response to the treatment; food would simply not improve their condition and subsequent singing behaviour. This is very unlikely due to the poor weather conditions, and since body condition was a non-significant variable on treatment.

In conclusion, experience seems to be only marginally significant on song repertoire and not significant on song rates. In general, experienced males displayed strong conservatism in which song figure types they sang and did not increase in repertoire size as a result of supplementary feeding. However, the experienced males that attained a mate this season did show an increase in repertoire size. What sets these individuals apart is not known. Increases in song rates were observed throughout the two groups, and is therefore not a consequence of treatment. Further studies are recommended to elucidate at which rate or by which means changes in song repertoires and song rates can be induced.

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